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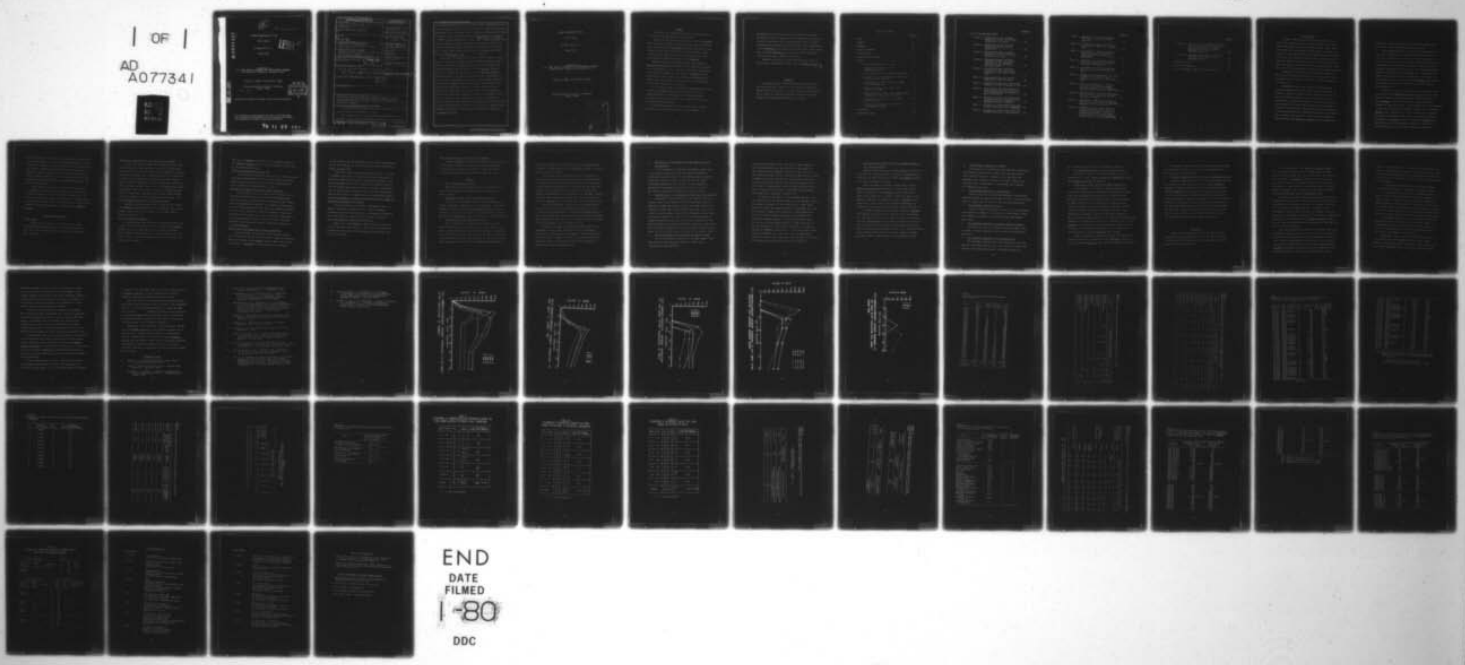

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KOREAN HEMORRHAGIC FEVER

Final Report

HO WANG LEE, M. D.

March 1979

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD 17-78-G-9444 & 9445

Korea University College of Mecicine
Seoul, Korea

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Korean hemorrhagic fever (KHF) was recognized for the first time in Korean in 1951 among US troops.

The investigator et al. reported the demonstration of a specific antigen and of antibodies of KHF in 1976, and went on

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to isolate the etiologic agent of KHF in 1978. Convalescent sera from hemorrhagic fever with renal syndrome in USSR, from epidemic hemorrhagic fever in Japan, from nephropathia epidemica in Finland and Sweden, have since been shown to be positive for antibodies.

This report presents the results on (1) isolation of (KHF) virus from patients (2) antibody responses in animals (3) the ratio of clinical and subclinical infection (4) cultivation of the virus in a tissue culture cells and (5) vertical transmission of KHF virus in Apodemus agrarius.

There were 379 hospitalized cases of KHF in Korea in 1978 and 10 of them were US Army soldiers. Twenty-one new strains of KHF virus from wild Apodemus and 8 strains of the same virus from KHF patients were isolated in normal Apodemus. Among 25 species of animals tested only tissues of Apodemus agrarius showed presence of the KHF virus antigen after inoculation of the virus by FA technique, while none of the animals showed any ill symptoms. Apodemus, Swiss albino mice, rats, guinea pigs, rabbits and monkeys were shown to produce specific IF and neutralizing antibodies against KHF virus after inoculation. Clinical and subclinical infection of KHF in the endemic areas were 0.5% and 0.5-1.4%, respectively. Out of 943 tested US military personnel stationed in Korea during years 1977 and 1978, five were sero-positive to KHF virus. Results of a limited study indicated that there is no vertical transmission of KHF virus in Apodemus agrarius.

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SUMMARY

There were 379 hospitalized cases of KHF in Korea in 1978 and 10 of them were US Army soldiers.

Twenty-one new strains of KHF virus from wild Apodemus were isolated in 1978; all tolled 114 strains of the viurs were isolated from wild Apodemus agrarius collected in the endemic areas of KHF from 1974 to 1978 inclusive. Eight strains of the same virus were also isolated from acute atage bloods of KHF patients in normal Apodemus.

Among 25 species of animals tested only tissues of Apodemus agrarius showed presence of the KHF virus antigen after inoculation of the virus by FA technique, while none of the animals showed any ill symptoms. Apodemus, Swiss albino mice, rats, guinea pigs, rabbits and monkeys were shown to produce specific IF and neutralizing antibodies against KHF virus after inoculation.

In a limitted study, clinical and subclinical infection of KHF in the endemic areas were 0.5% and 0.5-1.4%, respectively. Out of 943 tested US military personnel stationed in Korea during years 1977 and 1978, five were sero-positive to KHF virus.

It was confirmed that KHF virus grows well in human lung cancer cells (A549 cells) by the IFA method.

Antibodies of sera from patients with KHF, with epidemic hemorrhagic fever in Japan, and with nephropathia epidemica, as well as with sera from infected animals were titrated simultaneously against KHF virus antigen, which was prepared in both Apodemus lung tissues and in A549 cells. This showed that infected Apodemus lung tissues were more sensitive antigen system than infected A549 cells.

Results of a limitted study indicates that there is no vertical transmission of KHF virus in Apodemus agrarius.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animals, Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

Epidemic hemorrhagic fever was recognized for the first time in Korea in 1951 among US troops (1) and has since been known as Korean hemorrhagic fever (KHF). After the Korean War, the disease was designated as endemic in the area of the demilitarized zone (DMZ) between North and South Korea and since has been gradually spreading south-westerly. However, most cases are still reported near the DMZ where American and Korean soldiers are stationed. Korea-wide, 300 to 900 persons are hospitalized from KHF every year.

Hemorrhagic fevers with a very similar syndrome to KHF are being reported in Manchuria (2,3,4), Russia (5,6), Scandinavia (7,8), several countries in Eastern Europe (9) and Japan (10).

Although 60 years have passed since hemorrhagic nephroso-nephritis was first reported in Vladivostok, its etiologic agent and reservoir were unknown until 1975. In the early 1940's Japanese (2,3,4), and Russians (5,6), successfully reproduced hemorrhagic fever by injection of urine and sera of acute-stage patients into volunteers. Filtered sera of patients also produced clinical symptoms, so this disease had been suspected as being of viral origin. The injection into humans of a suspension of Trombicula mites obtained from Apodemus agrarius also reproduced the

hemorrhagic fever, such that mites had been suspected as the vector, and field rodents as the reservoir.

The investigator et al. reported the demonstration of a specific antigen and of specific antibodies of KHF in 1976 (11), and went on to isolate the etiologic agent of KHF in 1978 (12) for the first time. Convalescent sera from hemorrhagic fever with renal syndrome in USSR (12), from epidemic hemorrhagic fever in Japan (13), from nephropathia epidemica in Finland (14) and Sweden (15), have since been shown to be positive for the antibodies. In contrast, no fluorescence can be seen when infected Apodemus lung tissue is reacted with antisera to Marburg, Ebola and several arenaviruses (12).

From 1974 to 1978, 114 strains of the KHF virus were isolated from wild Apodemus agrarius coreae. The same virus was also isolated from acute bloods of 8 KHF patients in adult Apodemus. Virus-like particles, spherical with a diameter of about 60 nm and in crystalline array, were observed in the cell cytoplasms of pulmonary tissues from 5 infected Apodemus (16); confirmation of this finding is in progress by immune electronmicroscopy. The virus was successfully propagated in Apodemus through 30 passages; Apodemus ID_{50} was $10^{6.3}$/ml. The virus passed through a 0.1 μ but not a 0.05 μ millipore filter and was chloroform sensitive.

The infectivity of the virus was neutralized by convalescent serum. Experimentally inoculated Apodemus developed specific fluorescent antigen in lungs, kidneys, liver, parotid glands, and bladder. Antibodies appeared during the first week of symptoms, reached a peak at the end of the second week and have persisted as long as 14 years. Among residents of both non-endemic and endemic areas, antibody prevelance was 1.0% and 3.8% respectively.

This report presents the results of the project on; 1) isolation of KHF virus from Apodemus mice and patients; 2) antibody responses to the KHF virus in animals; 3) the ratio of clinical and subclinical infection in endemic areas; 4) cultivation of the virus in A549 cells and; 5) preliminary studies of vertical transmission of KHF virus in Apodemus agrarius.

MATERIALS AND METHODS

Survey areas

Surveys were carried out in Korean civilian farm villages where many cases of KHF have been reported each year. Two areas, namely Pochun and Songnaeri of Kyungido, were surveyed.

Collection, identification and processing of rodents

Using live traps, rodents were captured in the field, in uncultivated scrub vegetation, and near farm dwellings. Traps were set in the late afternoon and examined at midnight and at dawn. Animals were transported live to the laboratory in Seoul. Blood samples were obtained by cardiac puncture under chloroform anesthesia, and the animals were autopsied. Parotid glands, spleen, lungs, liver and kidneys were removed and weighed. Portions of each organ were titrated with BSS (pH 7.4) containing 1 percent bovine plasma albumin (BSSA) for virus isolation, and the remainder was stored at -70° C.

Normal Apodemus agrarius for laboratory studies

Apodemus agrarius coreae were obtained at Chin Island and Apodemus agrarius jejudoica were trapped on Jeju Island. Neither island has ever registered cases of KHF. These animals weighed 20-50 g.

Strain of KHF virus employed

All experimental and diagnostic work was done with lung tissues infected with KHF virus strain 76/118, 7th Apodemus passage (12). To titrate the infectious agent 10 percent lung suspensions were prepared with BSSA (clarified at 2,000 G for 20 min.), and supernatants were used to inoculate cell cultures and animals.

The ID_{50} in Apodemus was $10^{6.3}$/1.0 ml. Titration endpoints for Apodemus agrarius rodents were calculated 20 days later by the Reed-Muench method.

Fluorescent antibody technique (FAT)

The indirect FA procedure was used (12). FITC-conjugated polyvalent immunoglobulins of goat origin prepared against human, guinea pig, mouse, rat and rabbit immunoglobulins were purchased from the Hyland Co., Calif.

Specimens from KHF patients and normal human sera

Initial blood and urine samples were taken as soon as patients were hospitalized, and more blood samples were taken at regular intervals during the course of the illness. Sera from residents of endemic, near endemic and non-endemic areas of KHF were obtained from the local hospitals and kept at -40° C until used. Sera from US personnel in Japan who had never been to Korea were used as controls. Supernatants of 10% suspensions of lungs, kidneys and livers from KHF patients post mortem were used for isolation of KHF virus in normal Apodemus.

Animals for virus growth and antibody production

Helathy animals as listed in Table 12 were used for virus multiplication and for production of specific antibodies against KHF virus. 8,000 Apodemus ID_{50} of KHF virus (76/118, 7th passage in Apodemus was inoculated into the animals

intramuscularly and the antibody titers of sera were measured by the IFA technique at certain intervals after inoculation.

Tissue culture cells

A549 cells which were kindly supplied by Dr. G. French of USAMRIID were used for growth and antigen preparation of KHF virus. KHF virus 76/118 was inoculated into A549 cells, passed repeatedly at 15 day intervals and infected cells at the 7th passage were used as antigen. Details on the preparation of KHF antigen in A549 cells for the IFA technique are omitted here since Dr. French will soon publish them himself.

Sera from patients of epidemic hemorrhagic fever in Japan and nephropathia epidemica in Finland

Sera from patients of epidemic hemorrhagic fever in Sendai, Japan were obtained from Dr. Ishida of Tohoku University, and sera from patients of nephropathia epidemica were supplied by Dr. Lähdevirta of Helsinki University.

Sera from immunized animals with KHF virus

Immune sera from Apodemus, rabbits and rats produced in the experiment described above were used for comparative antibody titration with infected Apodemus lung tissues and A549 cell antigens.

Vertical tramsmission of KHF virus in Apodemus

Pregnant Apodemus infected with KHF virus were collected in the endemic areas and autopsied. Mothers, embryos and sucklings were examined for presence of KHF virus antigen by FAT for possible vertical transmission of KHF virus.

RESULTS

1. No. of KHF patients in 1978

There were 379 hospitalized cases of KHF patients in Korea in 1978 and 10 of them were US Army soldiers, as shown in Table 1.

2. Isolation of KHF virus from Apodemus agrarius

In 1978, frozen tissues from 135 rodents captured in endemic areas were examined by the IFA technique. 21 strains of KHF virus were isolated from Apodemus agrarius as shown in Table 2. A total of 114 strains of KHF virus were isolated solely from Apodemus mice during 1974-1978, as shown in Table 3.

3. Virus isolation from KHF patient blood

Ten to twenty ml of blood were taken from each KHF patient within 6 days after the onset of fever, and one week later a 2nd bleeding was performed. The increase of antibody titer to KHF virus in the 2nd blood specimen made it possible to confirm that these were KHF patients. 0.5 ml of the initial blood specimens were injected into Apodemus intramuscularly.

As shown in Table 4, the inoculation of 66 different blood specimens from KHF patients into Apodemus resulted in isolation of the virus in 8 cases.

Since specific IF antibodies appear in the patients blood right after onset of the disease, an attempt was made to isolate the virus from the acute serum free of antibodies, as was reported previously (12). Isolation of the virus was achieved in 2 cases out of 12 in 1976. In 1977 and 1978, without consideration as to the presence of antibody, 47 blood specimens were employed to isolate the virus by inoculating them into Apodemus; in 6 of these cases the virus was isolated.

The blood specimens in which the virus isolation was achieved were all collected within 6 days after the onset of illness. Two strains were isolated from antibody negative blood and another six strains from antibody positive specimens. This demonstrates that the virus is capable of being isolated from blood whether it has antibodies or not.

Attempts were made to isolate KHF virus from 11 urine specimens of early stage patients and also from autopsy materials of 5 KHF patients in Apodemus. These attempts were non-productive, as shown in Table 6. It was not possible to demonstrate KHF virus antigen in the lungs and kidneys of necropsy materials from KHF patients.

4. Appearence of IF antibodies to KHF virus in sera of KHF patients

It was necessary to know when antibodies against KHF virus appear in the sera of KHF patients for serologic diagnosis. As shown in Table 7, antibodies begin to appear right after onset of fever. In most but not all cases antibodies appeared by the 7th day. It was possible to demonstrate IF antibodies against KHF virus in sera of KHF patients after the 8th day from onset of illness.

5. Distribution of IF antibodies to KHF virus in human sera

Occurrence of IF antibodies to KHF virus in sera from endemic areas, near endemic areas and non-endemic areas of KHF near the DMZ and from US soldiers stationed in Korea was examined in 1978. The results showed that 3.8%, 2.7%, 1.0% and 0.5% of the samples contained IF antibodies, respectively, as shown in Tables 8,9,10 and 11. All of the sero-positive samples belonged in the age group of 20-50, and more of the positives were from females than males. Five out of the tested 943 US military personnel who were stationed in Korea during years of 1977 and 1978 were sero-positive, as shown in Table 8.

6. Ratio of clinical and subclinical infection of KHF

Out of 379 soldiers stationed in the KHF endemic areas near DMZ four had antibodies specific to KHF virus, constituting 1.1% positivity.

As shown in the Table 12 Mr. Chun and Mr. Kim, among the four antibody positives, had histories of admissions to the Capital Military Hospital because of KHF in 1975. In this limitted study clinical infection of KHF was 0.5%. Their antibody titers were 128 and 256, respectively. The other two men had no such histories and had low antibody titers of 64 and 32, respectively; these two may have had either a mild or subclinical infection of KHF which would indicate that subclinical infection is about 0.5%.

As shown in the Table 13, the antibody titers of the same population in the hyper-endemic area of KHF were tested two times, on October 22, 1977 and March 21, 1978, both before and after the epidemic season, to determine the ratio of clinical to subclinical infection of this populations. Out of 143 male soldiers stationed in the endemic area during one epidemic season, 2 sero-positives were found. The titers of IF antibodies specific to KHF virus were 32 and 64, respectively. During this period Oh, S. K. had no subjective symptoms, whereas Oh, S. K. was sick with common cold-like symptoms for 3 days. The ratio of subclinical infection of KHF in this group was 1.4% but ratio of clinical to subclinical infection was not able to calculate.

7. Multiplication of KHF virus and antibody responses to the virus in animals

Growth of KHF virus and antibody responses to the virus in animals was investigated by inoculating intramuscularly 8,000 ID_{50} of KHF virus (76/118 strain 8th Apodemus passage) into various species of animals.

Among 25 species of animals tested, only lungs of Apodemus agrarius showed presence of KHF virus antigen after inoculation of 8,000 Apodemus ID_{50} of KHF virus using the FA technique, as shown in Table 14. None of the animals showed any ill symptoms. However, Apodemus, Swiss albino mice, rats, guinea pigs, rabbits and monkeys all produced IF and neutralizing antibodies against the KHF virus.

Presence of KHF virus in various tissues of Apodemus was examined again at certain intervals after inoculation of 1,000 ID_{50} of the virus, as shown in Table 15. Viremia was first demonstrable 7 days after inoculation of the virus and it persisted for a few days. Virus multiplication was demonstrated in parotid glands as well as in liver and kidneys. It was noted 10 days after that immune complex coexisted with KHF virus in lung tissues for long period of time.

a) The antibody responses in rabbits:

The detection of fluorescent antibody after inoculating intramuscularly 8,000 ID_{50} of KHF virus into New Zealand white rabbits (Figure 1), showed that in two weeks the antibody titer reached a maximum of 256-8192. Then it was inclined to be in drop-down, in two months still holding for somewhat high level.

b) The antibody responses in guinea pigs:

Figure 2 pictures the antibody response in guinea pigs. Antibodies began to appear about day-20 after virus inoculation, rose to a peak titer of 128-512, and then declined slowly.

c) The antibody responses in rats:

Figure 3 shows the antibody responses in rats. Antibody first appeared 3 weeks after inoculation. The highest titers were observed generally at the 4th week and then dropped down slowly.

d) The antibody responses in Apodemus agrarius coreae:

As shown in Figure 4, antibodies began to appear after 2-3 weeks, reached a maximum titer and then declined slowly thereafter.

e) The antibody responses in Swiss albino mice:

The antibody responses were slow and poor in mice when compared with other animals. Only 2 mice out of 4 inoculated with KHF virus showed antibodies, and these lasted only

for a short period of time, as indicated in Figure 5.

f) The antibody responses in other various animals:

As shown in Table 14, antibody production was also demonstrated in mice, Rattus norvegicus, Apodemus peninsulae and Apodemus speciosus and monkeys.

8. Cultivation of KHF virus in tissue culture cells

Very recently, G. French of USAMRIID found that a continuous epithelial cell line-A549, derived from a human lung carcinoma-support multiplication of KHF virus as evidenced by specific fluorescence spots in the cytoplasm of these cells. We have confirmed his finding and have started to employ the use of A549 cells infected with KHF virus as antigen to measure IF antibodies to the virus. Simultaneous antibody titration of sera from patients of KHF, of epidemic hemorrhagic fever in Japan, and of nephropathia epidemica in Finland was carried out with KHF virus infected Apodemus lung tissues and with infected A549 cells. As shown in Table 16, infected A549 cells were not as sensitive an antigen system as infected Apodemus lung tissues.

Table 17 shows the results of comparative simultaneous antibody titration of immune sera from Apodemus, rabbit and rat with infected Apodemus lung tissue and infected A549 cells.

The results indicate that infected A549 cells with KHF virus can not be used as an antigen for demonstration of antibodies in sera of animals.

9. Vertical transmission of KHF virus in Apodemus agrarius

Limitted studies were done to research possible vertical transmission of KHF virus from infected mother Apodemus agrarius to embryos and sucklings. Five naturally infected pregnant Apodemus were caught in the endemic areas of KHF in 1978, their dates of infection unknown. Three of about 20 day-old embryos found in the uterus of one of the infected mothers were autopsied; the KHF antigen was not present in the lungs and other organs. The other four infected mothers had the virus in their lungs and kidneys but results with six of 19, two of 30, five of 38 and five of 67 day-old off'springs were negative, as shown in Table 18.

DISCUSSION

Among the 379 hospitalized cases of KHF in 1978, a serologic diagnosis was made in 10 of the cases involving US soldiers, in 168 cases with Korean soldiers and in 99 cases with Korean civilians.

It was reconfirmed that Apodemus agrarius coreae is the only known natural reservoir of KHF virus in endemic areas. During the past year, 21 new strains of the KHF virus were isolated from Apodemus, whereas the virus could not be found in the other 8 species tested. All tolled now, as the result of work carried on through the past five years (1974 to present), 114 strains of the KHF virus have been isolated exclusively from the Apodemus species.

In past years there have been reports that injection of hemorrhagic fever patient's blood and/or urine could induce the disease in volunteers. However, our efforts to isolate the virus from urine specimens were unsuccessful and furthermore, we can find no evidence to support or confirm the claim of successful propagation of the agent in experimental animals. Up to the present time, Apodemus agrarius remains the one and only animal which is susceptible to the KHF virus, as far as we can discern.

Our lab has isolated 8 strains of the virus from blood specimens of KHF patients; for the first time. In addition, we found that like specimens kept for prolonged periods at -60° C could also produce the same result, i.e. isolation of the virus. Infectivity of the isolated viruses towards Apodemus was neutralized completely with convalescent sera from KHF patients, as well as with immune rabbit sera.

However, we were not able to isolate the virus either from urine or from autopsy specimens of KHF patients, even though the Russians and Japanese have claimed they could reproduce the disease by inoculation of urine from acute-stage patients into volunteers.

IF antibodies to KHF virus were 100% demonstrable in the sera of patients 7 days after the onset of fever.

It was a surprise to find that antibodies specific to KHF virus were found in less than 4% of the residents of endemic areas, in spite of the fact that the disease has been recorded in these regions for the last 17 years. There is no doubt that in future years, cases of KHF will continue to occur in these endemic areas, since as of now only about 4% of the resident populations enjoy immunity to the disease.

It was demonstrated for the first time that among US soldiers stationed in endemic areas of Korea, there is an approximate 0.5% rate of subclinical infection; there is a 0.5% rate of subclinical infection among Korean soldiers stationed in the endemic areas as well.

Attempts to find a laboratory animal host for KHF virus study other than Apodemus agrarius have failed. However, several species of laboratory animals were found to produce antibodies after inoculation with the virus,

and these animals may now be used for production of immune sera. We now feel it necessary to find out whether these animals produce viremia and so may act as temporary reservoirs of KHF virus, whether this be as it occurs in nature, or be it perhaps a purely experimental, but nonetheless a potentially useful or even dangerous phenomena!

Recently, rather conclusive evidence was forwarded that the KHF virus was responsible for a series of outbreaks of KHF in the Japanese cities of Niigata, Sendai, Kobe and Nagoya, and further that the disease very probably was transmitted by urban rodents and laboratory rats. These recent findings cast a fresh epidemiological light on this disease, which hitherto has been regarded as basically rural.

A host system that can be readily adopted to manipulation in the laboratory is urgently needed and is urgently being sought. Recently we have succeeded in colonizing Apodemus agrarius jejudoica in the laboratory, but we can not anticipate use of the colonized Apodemus for experimental purposes, at least for the next several years, since very limitted numbers are available now.

We have confirmed Dr. French's observation that A549 cells support multiplication of KHF virus in vitro; this find has a certain logic to it and should come as no suprise,

inasmuch as the virus grows best in the lung alveolar cells of Apodemus agrarius in vivo, whereas A549 cells are themselves derived from human lung alveolar cells.

The A549 cells are a reasonably satisfactory detector system when used for assay of the virus and of the antibodies. However, our preliminary experiments have shown that A549 cells infected with KHF virus were not as sensitive as infected Apodemus lung tissues when used as antigen for demonstration and/or titration of antibodies to the virus, as visualized by the IF antibody technique.

Experiments as to the mode of transmission of the KHF virus in Apodemus agrarius in the laboratory animal are in progress now; a rather limitted attempt to demonstrate vertical transmission of the virus in pregnant Apodemus agrarius was unsuccessful. More careful and better organized experiments on vertical transmission of the virus are requested, such that a definative conclusion on this problem may be reached.

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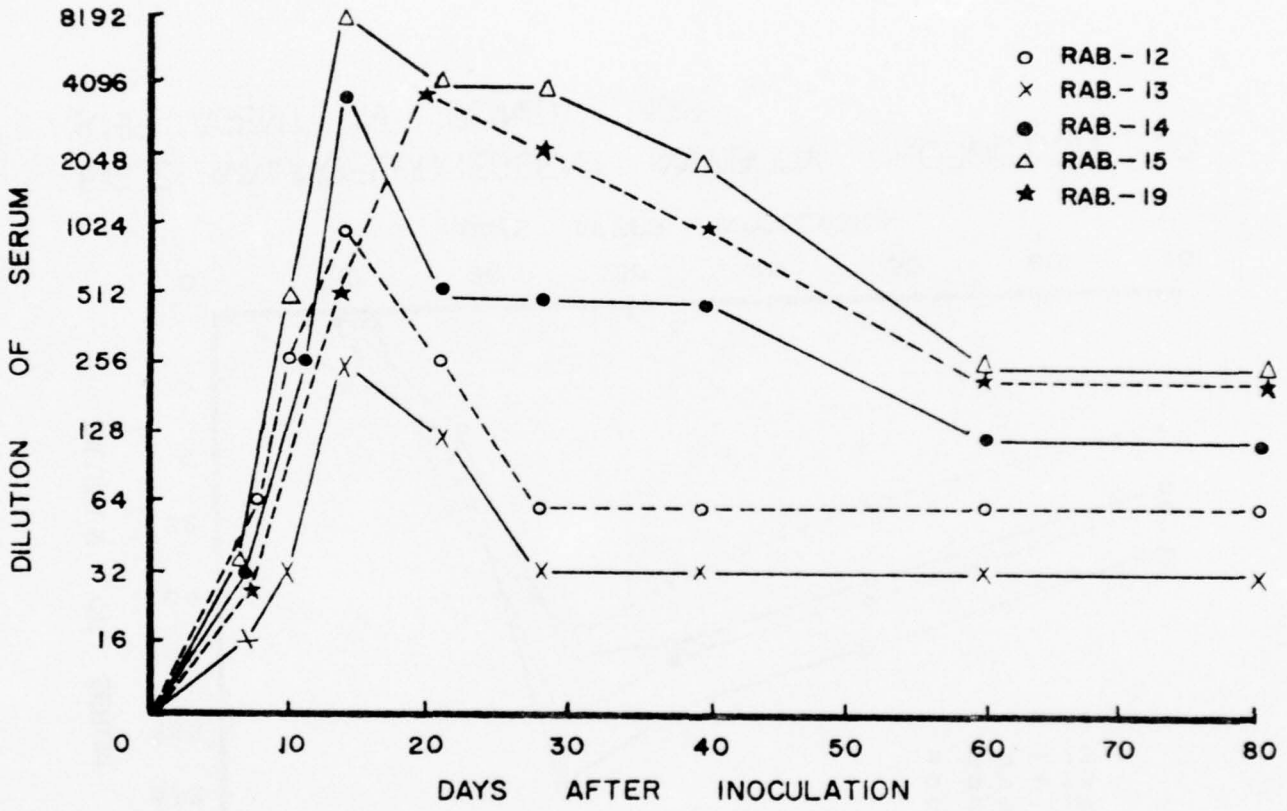

FIG. 1. IMMUNOFLUORESCENT ANTIBODY PRODUCTION TO KHF AGENT AFTER INOCULATION IN RABBITS

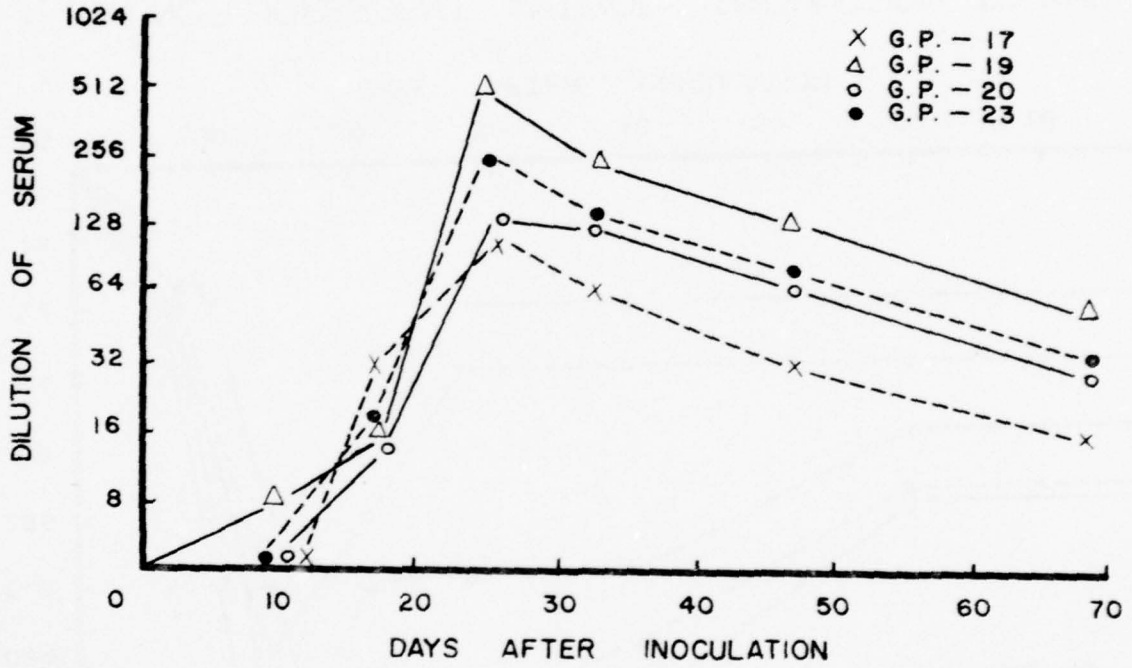

FIG. 2. IMMUNOFLUORESCENT ANTIBODY PRODUCTION TO KHF AGENT IN GUINEA PIGS

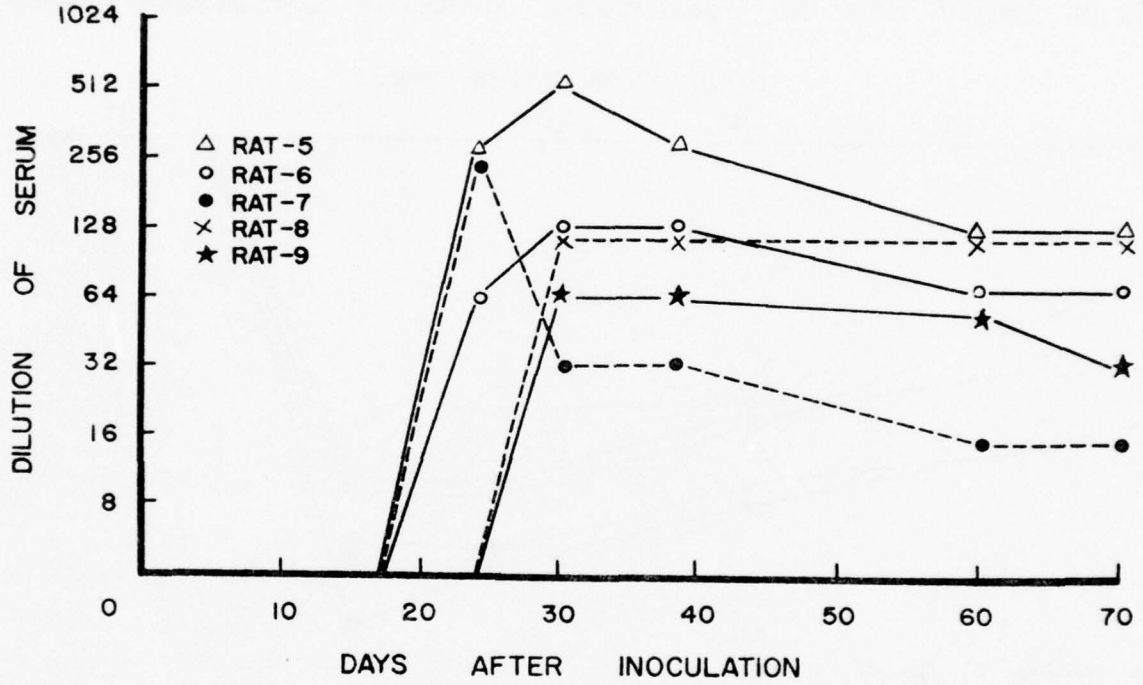

FIG. 3. IMMUNOFLUORESCENT ANTIBODY PRODUCTION TO KHF AGENT AFTER INOCULATION IN RATS

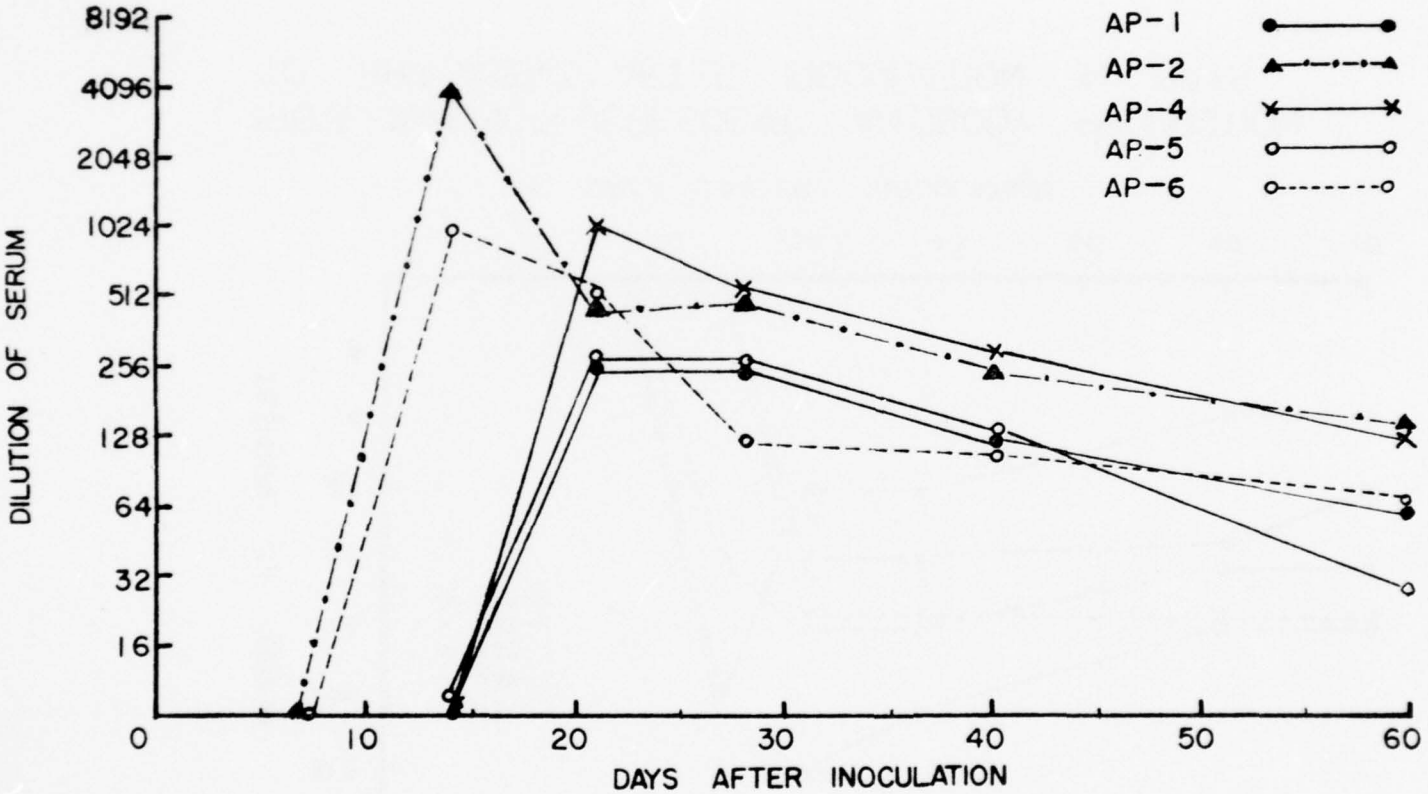

FIG. 4. IMMUNOFLUORESCENT ANTIBODY RESPONSES TO KHF AGENT AFTER INOCULATION INTO APODEMUS AGRARIUS COREAE

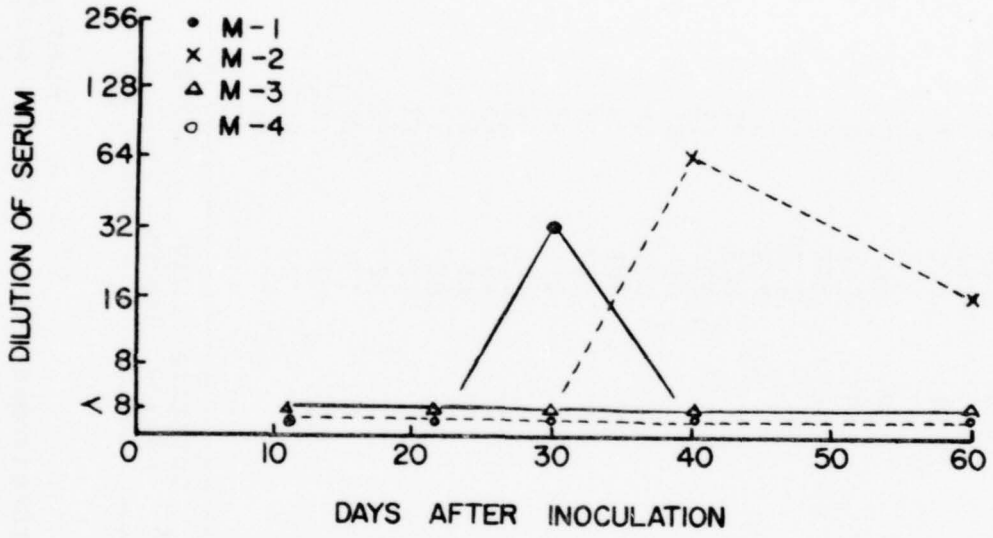

FIG. 5. IMMUNOFLUORESCENT ANTIBODY RESPONSES TO KHF VIRUS AFTER INOCULATION INTO SWISS-ALBINO MICE

Table 1
Hospitalized cases of Korean hemorrhagic fever patients

Year	US forces	Korean army	Korean civilian	Total
1951	827	...	...	827
1952	833	...	...	833
1953	455	...	...	455
1954	307	...	19	326
1955	20	...	...	20
1956	28	26	...	54
1957	13	21	...	34
1958	15	20	...	35
1959	79	47	...	126
1960	10	185	...	195
1961	27	341	...	368
1962	29	311	...	340
1963	11	257	...	268
1964	22	205	18	245
1965	99	110	2	211
1966	36	82	11	129
1967	31	86	13	130
1968	28	102	13	143
1969	9	134	8	151
1970	13	221	85	319
1971	2	358	311	671
1972	0	203	186	389
1973	0	237	241	478
1974	0	251	170	421
1975	1	370	466	837
1976	4	304	521	829
1977	7	212	288	507
1978	10	168	201	379
Total	2,916	4,251	2,553	9,720
Fatality	5%	7%	8%	6.6%

Table 2
Isolation of KHF virus from wild rodents collected by month in 1978

Species	Month and positives/tested												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Apodemus agrarius coreae			0/8		5/12	5/36		0/4	6/18	3/14	1/17	1/12	21/121
Mus musclus yamishinai						0/1		0/1	0/2		0/2	0/3	0/9
Crocidura lasiura									0/3				0/3
Clethrionomys rufocanus regulus											0/1		0/1
Micromys minutus ussuricus												0/1	0/1

Table 3
Isolation of the agent of Korean hemorrhagic fever (KHF) from wild small mammals captured in areas of Korea where KHF is endemic, by month (1974-1978)

Animal	No. of positive animals/no. tested												Total
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Apodemus agrarius coreae	3/46	0/19	1/72	5/40	19/86	11/107	1/52	4/31	35/98	23/136	9/89	2/41	114/817
(% positive)	(6.5)	(0)	(1.4)	(12.5)	(22.1)	(10.3)	(1.9)	(12.9)	(35.7)	(16.9)	(10.1)	(4.9)	(14.0)
Microtus fortis pelliceus	0/12	0/12	0/14	0/3	0/29	0/6	0/8	...	0/2	0/12	0/3	0/2	0/103
Crocidura lasiura	0/5	...	0/6	0/3	0/2	0/9	0/2	...	0/5	0/21	0/15	0/4	0/71
Clethrionomys rufocanus regulus	0/7	0/1	0/9	0/3	0/10	0/1	...	...	...	0/2	0/2	...	0/35
Cricetulus triton nestor	...	...	0/1	...	0/1	0/5	0/1	...	0/3	0/1	...	...	0/9
Mus musculus yamashinai	0/1	...	...	...	...	...	0/1	0/1	0/3	0/2	0/3	0/3	0/17
Micromys minutus ussuricus	0/1	...	0/1	...	...	...	...	...	...	...	...	0/1	0/3
Tamias sibiricus asiaticus	...	...	...	...	0/1	...	...	...	...	...	...	...	0/1

Table 4
Isolation of KHF virus from blood of KHF patient by inoculation into Apodemus agrarius jejudoica

Code of patient	Sex/ Age	Phase of illness	Day blood	Antibody titer	No. of infected / No. inoculated
76-109	M/21	Febrile	4	<8	4/8
76-242	M/21	"	2	<8	0/5
76-243	M/22	Oliguric	3	<8	2/7
76-253	M/21	Febrile	2	<8	0/3
76-259	M/21	"	3	<8	0/2
76-260	M/22	Oliguric	5	<8	0/5
76-267	M/22	Febrile	3	<8	0/4
76-270	M/23	"	5	<8	0/5
76-274	M/21	"	3	<8	0/4
76-288	M/22	"	2	<8	0/5
76-387	M/21	Oliguric	6	<8	0/5
77-137	M/23	"	4	512	9/10
77-184	M/21	"	6	4,096	0/3
77-216	M/22	Febrile	2	<8	0/3
77-219	M/22	Diuretic	3	128	0/3
77-231	M/22	Oliguric	5	512	0/3
77-232	M/21	Diuretic	6	2,048	0/3
77-256	M/23	Febrile	3	32	0/3
77-257	M/22	Diuretic	4	4,096	0/3
77-258	M/23	"	6	4,096	0/3
77-259	M/21	Febrile	4	512	5/7
77-299	M/26	Oliguric	4	4,096	0/3
77-300	M/22	"	5	4,096	0/3
77-308	M/23	Diuretic	4	512	0/2
77-309	M/21	Febrile	3	512	0/3
77-332	M/21	"	3	512	0/3
77-333	M/22	"	3	<8	2/6
77-335	M/23	"	3	2,048	5/6
77-350	M/22	Diuretic	5	4,096	0/3
77-351	M/22	Febrile	3	2,048	0/3
78-105	M/21	"	3	1,024	0/4
78-151	M/21	Oliguric	4	1,024	0/3
78-182	M/22	Febrile	5	256	0/5
78-199	M/22	Oliguric	4	512	0/6
78-200	M/22	Diuretic	4	1,024	0/6
78-228	M/22	Febrile	5	512	0/6
78-229	M/23	"	4	512	0/6
78-230	M/22	Diuretic	4	512	0/6

continued

78-231	M/21	Oliguric	5	1,024	0/7
78-232	M/23	"	6	512	0/6
78-233	M/23	"	4	1,024	0/6
78-267	M/21	Febrile	3	<16	0/3
78-273	M/22	Diuretic	4	<16	0/6
78-275	M/22	Hypotensive	4	<16	0/6
78-277	M/23	Febrile	4	<16	0/4
78-296	M/31	"	4	1,024	0/3
78-302	M/22	Diuretic	3	512	0/3
78-318	M/21	Hypotensive	6	128	3/6⇓
78-319	M/22	Diuretic	6	128	0/3
78-320	M/23	"	4	256	0/3
78-321	M/22	"	6	128	0/3
78-276	M/22	"	5	512	0/6
78-281	M/22	Oliguric	4	128	0/6
78-282	M/22	Febrile	5	512	0/6
78-284	M/22	"	5	128	0/3
78-285	M/21	Diuretic	4	1,024	0/3
78-286	M/23	"	4	512	0/5
78-346	M/23	"	6	<16	0/5
78-348	M/28	Hypotensive	4	256	2/6⇓
78-349	M/23	Oliguric	5	1,024	0/3
78-350	M/22	"	5	256	0/3
78-371	M/22	"	6	256	0/2
78-372	M/21	Febrile	4	64	0/3
78-387	M/22	"	3	128	0/5
78-388	M/23	"	4	1,024	0/5
78-389	M/21	"	3	1,024	0/5

⇓ : Repeated with original blood specimens that were kept in -60° C for 10 - 16 months and confirmed the results.

$$\frac{\text{Total No. of KHF virus isolated}}{\text{Total No. of blood specimen tested}} = \frac{8}{66}$$

Table 5
Attempts to isolate KHF virus from urines of KHF patients in *Apodemus*

No.	Code No. of patient	Day urine	No. of *Apodemus* infected/no. tested
1	77-46	3	0/3
2	77-48	5	0/3
3	77-52	4	0/3
4	78-275	4	0/3
5	78-276	5	0/3
6	78-277	4	0/3
7	78-281	4	0/3
8	78-282	5	0/3
9	78-284	5	0/3
10	78-285	4	0/3
11	78-286	4	0/3

TABLE 6
ATTEMPTS TO ISOLATE KHF VIRUS FROM AUTOPSY MATERIALS OF KHF PATIENTS

Code of patient	FA antibody titer of serum of patients against KHF agent	Tissue examined	FA staining of the tissue for Korea antigen	No. infected / No. of Apodemus inoculated
KHF76162	256	Lungs	-	0/3
		Kidneys	-	0/3
KHF76191	2,048	Lungs	-	0/3
KHF76194	4,096	Lungs	-	0/3
		Kidneys	-	0/3
KHF76403	128	Lungs	-	0/3
		Kidneys	-	0/3
		Liver	-	0/3
KHF76SNU	4,096	Lungs	-	0/3
		Kidneys	-	0/3

TABLE 7
APPEARANCE OF IF ANTIBODIES TO KHF VIRUS
IN THE SERA OF KHF PATIENTS

	DAYS AFTER ONSET OF FEVER									
	1	2	3	4	5	6	7	8	9	10
NO. POSITIVE	6[1]	15	37	64	59	32	28	27	12	24
NO. TESTED	7[2]	20	48	67	63	34	30	27	12	24
(% POSITIVE)	(86)	(75)	(77)	(96)	(94)	(94)	(93)	(100)	(100)	(100)

[1] : Positive indicates antibody titer over 16.

[2] : All of the 332 patients were confirmed as KHF by test.

Table 8
Incidence of immunofluorescent antibodies to KHF virus in human sera

Group	Antibody to KHF Virus No. of Positive / No. of Tested (%)
Resident of hyper-endemic area, Tongduchun	6/148 (4.0)
Resident of near endemic area, Chunchun	2/73 (2.7)
Resident of non-endemic area, Seoul	1/132 (0.8)
Military personnel (U.S. Army)	5/943 (0.5)

TABLE 9
OCCURRENCE OF IMMUNOFLUORESCENT ANTIBODIES AGAINST KHF VIRUS AMONG RESIDENT OF ENDEMIC AREA, TONGDUCHUN

Age Group	Sex	No. Tested	No. Positive / Total No. Tested
1 - 10	F	3	0/6
	M	3	
11 - 20	F	2	0/6
	M	4	
21 - 30	F	35(2)*	3/55
	M	20(1)	
31 - 40	F	15(2)	3/37
	M	22(1)	
41 - 50	F	8	0/28
	M	20	
51 > 51	F	0	0/16
	M	16	
Total	F	63(4)	6/148 (4.0%)
	M	85(2)	

* ( ): No. of positive

TABLE 10
OCCURRENCE OF ANTIBODIES AGAINST KHF VIRUS
AMONG THE RESIDENT OF CHEUNCHUN, KWANGWON-DO

Age Group	Sex	No. Tested	No. Positive / Total No. Tested
1 - 10	F	2	0/3
	M	1	
11 - 20	F	23	0/35
	M	12	
21 - 30	F	4	1/11
	M	7(1)*	
31 - 40	F	2	0/6
	M	4	
41 - 50	F	4(1)	1/7
	M	3	
51 - 60	F	3	0/7
	M	4	
> 61	F	0	0/4
	M	4	
Total	F	38(1)	2/73 (2.7%)
	M	35(1)	

* ( ) : No of positive

TABLE 11
OCCURRENCE OF ANTIBODIES AGAINST KHF VIRUS
AMONG THE RESIDENT OF SEOUL

Age Group	Sex	No. Tested	No. Positive / Total No. Tested
1 - 10	F	8	0/18
	M	10	
11 - 20	F	3	0/7
	M	4	
21 - 30	F	11	0/18
	M	7	
31 - 40	F	10	0/31
	M	21	
41 - 50	F	8	0/25
	M	17	
51 - 60	F	7 (1)*	1/20
	M	13	
> 61	F	6	0/13
	M	7	
Total	F	53 (1)	1/132 (0.8%)
	M	79	

* ( ) : No of positive

Table 12
Appearent and inappearent infection of KHF virus among military personnel stationed in the endemic area

$$\frac{\text{No. of Positive}}{\text{Total No. of Tested}} = \frac{4}{378} = 1.1\%$$

No.	Name	Antibody Titer to KHF Virus	History of Clinical KHF
1	Chun, J. J.	128	Hospitalized with severe symptoms of KHF on November 1975
2	Kim, S. H.	256	Hospitalized with severe symptoms of KHF on December 1975
3	An, S. H.	64	No
4	Park, C. W.	32	No

Table 13
Ratio of subclinical to clinical infections of KHF virus among soldiers stationed in Tongduchun during epidemic season of 1977-1978

Antibodies to KHF Virus	Date of Bleeding	
	1st Bleeding 10/22/77	2nd Bleeding 3/21/78
No.of Positive / No.of Tested	0 / 143	2* / 143

Name	Antibody Titer to KHF Virus 10/22/77	3/21/78	Clinical Symptoms During the Period
Oh, S. N.	0	32	None
Oh, S. K.	0	64	Flu Symptoms on 1/25-28/78

* :

Table 14
Multiplication of Korean hemorrhagic fever virus and antibody response in animals

Species	No. infected / No. inoculated	Clinical signs	Antibody response
C-DBA/2N mice	0/10	-	-
Suckling hamster, Syrian	0/8	-	-
Adult hamster, Syrian	0/17	-	-
Hamster, Chinese	0/4	-	-
Mongolian Gerbil	0/6	-	-
Cricetulus triton nestor	0/18	-	-
Microtus fortis pelliceus	0/4	-	-
Clethrionomys rufocanus regulus	0/4	-	-
Tamias sibiricus	0/3	-	-
Mus musculus	0/6	-	-
.....			
Suckling albino mice	0/88	-	+
Adult albino mice	0/7	-	+
C57 BL/6 mice	0/14	-	+
C_3H mice	0/4	-	+
Rat/S.D.	0/15	-	+
Rat/Wister	0/20	-	+
Rattus norvegicus	0/16	-	+
Guinea pigs	0/23	-	+
Rabbit, Newzealand	0/9	-	+
Squirrel monkey	0/3	-	+
Cynomologus monkey	0/3	-	+
Rhesus monkey	0/3	-	+
Apodemus peninsulae giliacus	0/4	-	+
Apodemus speciosus	0/3	-	+
Apodemus agrarius coreae	42/50	-	+
Apodemus agrarius jejudoica	46/46	-	+
Apodemus agrarius ningpoensis	3/3	-	+

Table 15
Distribution and multiplication of KHF virus in the tissues of Apodemus agrarius after inoculation

Virus strain, dose and route of inoculation	Tissue	Presence of KHF virus days after inoculation									
		3	5	7	10	13	21	34	50	76	100
76/118/AP8 1,000 Apodemus ID_{50} intramuscular	Blood	-[1] 0/3[2]	- 0/3	+ 3/3	+ 3/3	- 0/3	- 0/3	- 0/3			
	Lungs	- 0/3	- 0/3	- 0/3	++++ 3/3	+++ 2/3	+++ 3/3	+ 2/3	+ 2/3	+ 2/3	+ 2/3
	Kidneys	- 0/3	- 0/3	- 0/3	+++ 3/3	+ 2/3	+ 3/3	+ 1/3	+ 1/3	+ 1/3	+ 1/3
	Liver	- 0/3	- 0/3	- 0/3	++ 3/3	+ 2/3	+ 3/3	+ 1/3	+ 1/3	+ 1/3	+ 1/3
	Parotid glands	- 0/3	- 0/3	- 0/3	+ 3/3	+ 2/3	+ 2/3	+ 1/3	+ 1/3	+ 1/3	+ 1/3
	Bladder	- 0/3	- 0/3	- 0/3	+ 3/3	- 0/3	- 0/3	- 0/3	+ 1/3	- 0/3	- 0/3
	Spleen	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3
Immune complex	Lungs	- 0/3	- 0/3	- 0/3	++ 3/3	++ 2/3	++ 3/3	+ 1/3	++ 2/3	++ 2/3	+ 3/3

[1] : The fluorescent reaction was graded as - or + (from + to ++++).
[2] : No. of animals infected/no. tested.

Table 16
Comparative IF antibody titration of sera from patients of KHF, of Japanese hemorrhagic fever and of nephropathia epidemica with KHF virus antigen of infected Apodemus tissues and of infected A549 cells

Disease/ serum No.	Antibody titer to KHF virus antigen of Apodemus lungs (No. positive/no. tested)		A549 cells	
KHF/78-416	4,096		32	
KHF/78-417	1,024		32	
KHF/78-418	1,024		16	
KHF/78-419	1,024		<16	
KHF/78-420	1,024		16	
KHF/78-423	256		16	
KHF/78-431	4,096		64	
KHF/78-432	256		64	
KHF/79-21-3	64	(18/18)	32	(11/18)
KHF/79-22	128		<16	
KHF/76-21	2,048		256	
KHF/79- 5	1,024		<16	
KHF/79- 9	256		<16	
KHF/79-21-5	4,096		128	
KHF/79-45	256		<16	
KHF/79-53	8,192		256	
KHF/79-54	8,192		<16	
KHF/79-56	512		<16	
EHF/41	128		128	
EHF/57	128		32	
EHF/59	128		32	
EHF/75	32		<16	
EHF/81	64		<16	
EHF/82	32		<16	
EHF/Y1	2,048	(14/14)	64	(5/14)
EHF/Y2	128		<16	
EHF/Y3	64		<16	
EHF/Y4	128		<16	
EHF/Y5	256		<16	
EHF/Y6	64		<16	
EHF/Y7	64		<16	
EHF/P2	256		256	

NE/ 4	32		<16	
NE/ 5	32		<16	
NE/ 6	32		32	
NE/10	64		64	
NE/18	32		<16	
NE/20	32		32	
NE/21	32		<16	
NE/29	32	(15/15)	32	(9/15)
NE/32	32		32	
NE/33	128		128	
NE/35	32		<16	
NE/36	32		32	
NE/46	128		32	
NE/47	128		<16	
NE/50	32		32	

KHF: Korean hemorrhagic fever
EHF: Epidemic hemorrhagic fever, Japan
NE : Nephropathia epidemica, Finland

Table 17
Comparative IF antibody titration of sera from infected animals with KHF virus antigen of infected Apodemus lung tissues and of infected A549 cells

Animal/ serum No.	Antibody titer to KHF virus antigen of Apodemus lungs (No. positive/no. tested)		A549 cells	
Apodemus/13-2	256		32	
Apodemus/13-3	256		32	
Apodemus/13-4	256		<16	
Apodemus/14-1	256		32	
Apodemus/14-2	256		32	
Apodemus/14-3	32	(12/12)	<16	(8/12)
Apodemus/15-1	1,024		256	
Apodemus/15-2	256		256	
Apodemus/15-3	32		<16	
Apodemus/79-102	128		<16	
Apodemus/79-104-1	128		64	
Apodemus/79-104-2	128		64	
. . . . .				
Rabbit/ 9	128		<16	
Rabbit/10	128		<16	
Rabbit/26	64		<16	
Rabbit/28	128		<16	
Rabbit/30	256	(9/9)	<16	(2/9)
Rabbit/45	2,048		64	
Rabbit/46	1,024		64	
Rabbit/47	1,024		<16	
Rabbit/48	1,024		<16	
. . . . .				
Rat/wak- 1	1,024		256	
Rat/wak- 2	32		32	
Rat/wak- 3	2,048		256	
Rat/wak- 4	64		<16	
Rat/wak-25	2,048	(8/8)	256	(7/8)
Rat/wak-26	1,024		128	
Rat/wak-34	512		32	
Rat/wak-37	2,048		1,024	

TABLE 18

ATTEMPTS TO DEMONSTRATE VERTICAL TRANSMISSION OF KHF VIRUS IN APODEMUS AGRARIUS

Pregnant Apodemus			Embryo		
Code No. & place of collection	KHFV in lungs	IF antibodies to KHFV	No.	Day-old	KHFV in lungs
R76/175 Songnaeri	++	-	1	20	-
			2	20	-
			3	20	-

Mother Apodemus			Baby Apodemus			
Code No. & place of collection	KHFV in lungs	Viremia	No.	Days after birth	KHFV in lungs	IF antibodies to KHF virus in serum
R76/305 Songnaeri	+++	n.t	1	19	-	-
			2	19	-	-
			3	19	-	-
			4	19	-	-
			5	19	-	-
			6	19	-	-
R76/Y Yeoju	+++	n.t.	1	30	-	-
			2	30	-	-
KR/17 Keojedo	+++	-	1	38	-	-
			2	38	-	-
			3	38	-	-
			4	38	-	-
			5	38	-	-
KR/18 Keojedo	++	-	1	67	-	-
			2	67	-	-
			3	67	-	-
			4	67	-	-
			5	67	-	-

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LIST OF PUBLICATIONS

1. Lee, H. W., Lee, P. W. & Johnson, K. M.: Isolation of etiologic agent of Korean hemorrhagic fever. J. Infect. Dis., 138: 298-308, 1978.

2. Lee, H. W.: Korean hemorrhagic fever: Ebola virus Hemorrhagic Fever, Elsevier/North-Holland Biomedical Prass, 331-343, 1978.

LIST OF PERSONNEL RECEIVING GRANT SUPPORT

1. Lee Pyung Woo Ph.D. Virologist, Fluorescent and Immune Electron Microscopic Work.

2. Liu Sun Ja. Tissue Culture Technician.

3. Kim Soo Am. Field Worker.

4. Im Yong Muk. Driver and Field Worker.

5. Han Hyung Choon. Animal Caretaker.

128		<16	
128		<16	
64		<16	
128		<16	
256	(9/9)	<16	(2/9)
048		64	
024		64	
024		<16	
024		<16	
024		256	
32		32	
048		256	
64		<16	
048	(8/8)	256	(7/8)
024		128	
512		32	
048		1,024	